SUPPLEMENTARY INFORMATION

RESULTS AND DISCUSSION

The *secY40* mutation does not interfere with the stability of SecY

In order to exclude the possibility that the *secY40* mutation influences the stability of SecY we performed western blot analyses. The data shown in Fig. S1 demonstrate the presence of equal amounts of SecY in wild type and *secY40* INV. As negative control we used INV from *E. coli* strain CM124. In this strain SecE is depleted in the absence of arabinose, which concomitantly leads to increased proteolysis of SecY. In summary, the *secY40* mutation does not influence the stability of SecY, but specifically interferes with the integration of SRP-dependent membrane proteins.

METHODS

Plasmid construction

pET19b-YidC, expressing YidC containing a His_{10}-tag under the control of the T7 promoter was constructed by PCR-amplifying the YidC region of pROEX-Htb-YidC (Samuelson *et al.*, 2000) using the primer NdeIYidC (5'-CAGGGCCATATGGATTCGCA-3') and XhoIYidC (5'-GCGCATGCCTCGAGACTGCA-3'). The PCR product obtained was digested with NdeI and XhoI and ligated into the NdeI/XhoI digested vector pET19b (Novagen). pET19b-FtsY was obtained by ligating the 1.5 Kb NdeI/BamHI fragment of pET9a-FtsY (Luirink *et al.*, 1994) into NdeI/BamHI-digested pET19b. To construct the

plasmid pET22b-FtsY307, the AN-domain encoding segment of pET19b-FtsY was PCR amplified using the primer FtsY307fw (5'-AGATCTCGATCCCGCGAAATT-3') and FtsY307rev (5'-GCCGAAGCTTCATCATCATCATGGATCCGGTTTTACCCACAC-CGTTG-3'). The amplified fragment was NdeI/HindIII digested and cloned into the corresponding sites of pET22b (Novagen). All constructs were confirmed by DNA sequence analyses.

Protein purification

For purification of FtsY307, pET22b-FtsY307 was transformed into Tuner^{TM} (DE3) pLysS (Novagen) and expression was induced in LB medium with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) at an OD600 of 0.5. Cells were grown for an additional 2 h at 37°C, collected by centrifugation and resuspended in buffer P (50 mM Hepes/KOH, pH 7.6, 1 M NH_4Cl, 10 mM $MgCl_2$ and 7mM ß-mercaptoethanol). Cells were disrupted with a French pressure cell, insoluble material was removed by centrifugation (30 min at 13000 rpm) and the supernatant was applied to a Talon metal affinity resin (BD Biosciences). After washing the resin with 20 bed volumes of buffer P, the resin was filled into a 2 ml gravity-flow column (Qiagen) and proteins were eluted with a 10-200 mM imidazole gradient in buffer P. The eluted proteins were analyzed on SDS-gels and pure FtsY-307 fractions were dialyzed against buffer F (50 mM Hepes/KOH, pH 7.6; 10 mM KCl, 10 mM $MgCl_2$ and 7 mM ß-mercaptoethanol) and stored at -80°C.

For the isolation of membrane-bound FtsY, pTP37, carrying FtsY-His_6 in pET22b (Powers & Walter, 1997) or pET9a-FtsY, carrying FtsY without His-Tag (Luirink *et al*., 1994) were transformed into Tuner^{TM} (DE3) pLysS (Novagen) and expression was induced in LB medium with 0.5 mM isopropyl-β-D-thiogalactoside (IPTG) at an OD600 of 0.5. Cells

were grown for an additional 2 h at 37°C, collected by centrifugation and resuspended in buffer A (50 mM Triethanolmanine-acetate pH 7.5, 250 mM sucrose, 1mM DTT, 1mM EDTA, 0.5 mM PMSF). Cells were disrupted with a French pressure cell, insoluble material was removed by centrifugation (30 min at 13000 rpm) and the supernatant subjected to an ultracentrifugation step (2 h, 150 000 g, 4°C). FtsY with or without His_6 tag at the C-terminus was purified from crude membranes by solubilization with 1% dodecyl-ß-D-maltoside (DDM) in 50mM Tris/HCl pH 8.0, 20% glycerol, 0.5 mM PMSF in the presence of the Complete-protease inhibitor cocktail (Roche). After centrifugation (55 000 rpm, 4°C, for 30 min in a Beckmann TLA 100.2 rotor), the supernatant was incubated with 0.5 ml of Talon™ resin in solubilization buffer containing 250mM NaCl. After washing, proteins were eluted by increasing the imidazole concentration from 10 to 200 mM (in 20% glycerol, 0.02 % DDM). Fractions were analyzed by Coomassie blue staining and western blot analyses using anti-SecY and anti-YidC antibodies.

Chemical crosslinking

Chemical crosslinking was performed as follows: SecY was *in vitro* synthesized in the presence of INV and the INV were subsequently isolated by centrifugation (55 000 rpm, 4°C, for 30 min in a Beckman TLA100.3 rotor). To remove membrane bound FtsY, the INV were treated with 4M urea in 50 mM Hepes/KOH, pH 7.5 for 1 h at 4°C. After washing the INV twice with INV buffer (50 mM Hepes/KOH, pH 7.5; 0.25 M sucrose), INV were resuspended in INV buffer, before incubating them with either buffer or cytosolic extracts derived from an FtsY-depleted strain (E. coli N4156 pAra14-FtsY'; Luirink *et al.,* 1994) or an FtsY-containing strain (Tuner (DE3) pLysS pET9a-FtsY, Luirink *et al.*, 1994). After incubation for 15 min at 37°C, the soluble, lysine-specific cross linker BS^3 was added at a final concentration of 1mM and the samples were incubated for 30 min at 4°C before the

crosslinker was quenched by the addition of 20 mM Tris/HCl pH 7.5. If not continued by immunoprecipitations, samples were precipitated with 5% TCA and prepared for SDS-PAGE. Immunoprecipitations were performed with a 6-fold scaled up reaction using polyclonal rabbit anti-FtsY and anti-SecY antibodies, bound to protein A-Sepharose matrix.

REFERENCES

Powers T, Walter P (1997) Co-translational protein targeting catalyzed by the *Escherichia coli* signal recognition particle and its receptor. *EMBO J.* **16**: 4880-4886

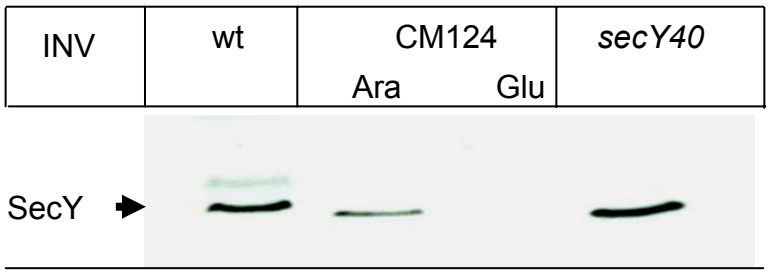

Fig. S1: The *secY40* mutation does not interfere with the stability of SecY
The SecY content in INV derived from wild type and *secY40* cells was determined by western blot analyses. As control, INV from strain CM124 were used in which the expression of the *secE* gene is under arabinose control.